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REVIEW

Neuroendocrinology of Osmoregulation in Crabs

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ABSTRACT—Neuroendocrine factors regulate water and salt movements in hyperregulating crabs. Two factors are found in the thoracic ganglion and hemolymph. A water soluble factor decreases water influx while an acetone soluble factor increases influx into the animal via the posterior gills.

Dopamine, found in the pericardial organ, and cAMP increase sodium uptake in the gill. Additionally, these compounds increase Na^+K^+ATPase activities of the gills. Na^+K^+ATPase is an adaptive enzyme, responding to decreases in the environmental salinity. Exposure of crabs to dilute salinity also causes increases in heart rates in response to cardioexcitors of the pericardial organ. All of these activities are involved in the integration of osmoregulation in crabs.

INTRODUCTION

Crabs (Brachyura, Decapoda), as with other crustaceans, inhabit various habitats from the deep sea through intertidal and estuarine waters into freshwater and terrestrial habitats and encounter a variety of osmoregulatory problems. Various crabs may be stenohaline in nature and be restricted to narrow saline environments. Others are euryhaline and can accomodate a wide range of salinities ranging from seawater to freshwater. In euryhaline crabs which migrate freely from a hypersaline seawater environment into freshwater streams as the blue crab *Callinectes sapidus* or the mud or mangrove crab *Scylla serrata*, it appears obvious that considerable osmotic adaptations would be necessary and that these adaptations would be controlled by hormones. This paper will attempt to review the evidences for the involvement of neurohormones in the regulation of salt and water balance in euryhaline, hyperregulating crabs.

Early work on neurosecretion and osmoregulation in crustacens had demonstrated that neuroendocrine systems as the X-organ sinus gland system, brain and thoracic ganglion may be involved in maintaining salt and water balance. Studies were concerned primarily with changes in weight and salt and water contents of the hemolymph, especially in relation to the molt cycle [1]. Neuroendocrine control of hydromineral homeostasis in crustaceans was first suggested by McWhinnie [2] in 1962 by her studies on the regulation of calcium levels in the hemolymph of the freshwater crayfish. She injected extracts of neuroendocrine tissues into the crayfish which affected changes in the free calcium levels in the hemolymph of intermolt animals. This led to a number of studies on neuroendocrine regulation of salt and water balance among crustaceans which have been reviewed [3, 7]. There are increasing evidences that neuroendocrine factors, amines and peptides, affect osmoregulation, especially among the decapod crustaceans, crabs, lobsters and shrimps. Evidences include the presence of factors which regulate water and salt movements in the gills, the role of the cyclic nucleotide cAMP as a second messenger, the effect of osmotic stress and dopamine on the gill sodium pump enzyme Na^+K^+ATPase, and the effect of the pericardial organ cardio excitor hormones on osmoregulation among crabs.

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Most crabs can be classified as osmoregulators or osmoconformers. Osmoregulatory capacities of five crabs found in Hawaiian waters are presented in Fig. 1. Chloride concentration of the hemolymph, a good indicator of total osmotic concentration is plotted against percent seawater concentration. The bold line represents isotonicity, the chloride concentrations being equal in the hemolymph and seawater (SW). Concentrations of chloride in hemolymph to the left of isotonicity would represent hyperregulation with hyporegulation being represented by hemolymph concentrations to the right of the isotonic line. Hemolymph concentrations of osmoconformers as *Calappa hepatica* and *Podophthalmus vigil* conform to that of the medium. Excellent regulators as *Metopograpsus thukuhar* can hyperregulate in dilute media below 70% SW and hyporegulate above that SW concentration. *Portunus sanguinolentus* is a good hyporegulator while *Thalamita crenata* is a good hyperregulator. Most studies on crustacean osmoregulation have been devoted to hyperregulation and it is this hyperregulation which appears to be regulated by neurohormones.

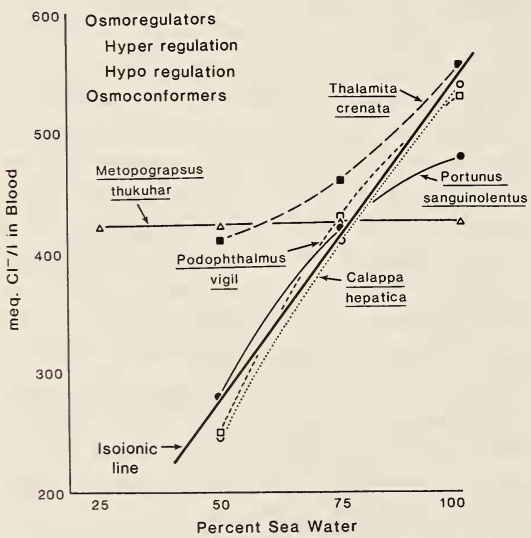

Fig. 1. Osmoregulatory capacities of five Hawaiian crabs. Modified from Kamemoto and Kato [8].

Neurohormones and Water and Salt Movement

Brain and thoracic ganglion (ThG) factors regulate the movement of tritiated water into the animal from the external medium in *Thalamita crenata* [9]. ThG was extracted with acetone to remove the acetone soluble factor. ThG was then extracted with deionized water. The water soluble fraction, injected into intact crabs, caused a decrease in the influx of tritiated water by 70% when compared to control, saline injected animals. This water soluble, influx decreasing factor is believed to be a peptide with a molecular weight of 800–1000 daltons on the basis of sephadex separations. A second factor, a yet to be described acetone soluble factor, caused an increase in the influx of water by 19% when compared to controls, indicating the presence of antagonistic factors regulating the influx of water into crabs.

Gills appear to be a primary site of action for the two factors. Crabs were clamped dorsal side down and only the gill chambers were irrigated with SW containing tritiated water. Hemolymph was collected and analyzed for the influx of tritiated water. Injection of the water soluble factor caused a 42% decrease in the influx of water while the acetone soluble factor caused a 16% increase in influx.

The posterior gills are believed to be the target tissue for the two factors. The posterior gills are known to be the sites of major osmoregulatory activity, the regulation of monovalent ions and more specifically of sodium uptake [10–15]. Specialized cells of the posterior gills are rich in mitochondria and have high levels of Na^+K^+-ATPase, basic characteristics of sodium regulating cells [15–21].

The posterior gills have been isolated and perfused effectively to study the roles of extracts on water and sodium movements. Tullis [22] developed a technique for the perfusion of an isolated crab gill, cannulating the afferent and effecrent vessels with polyethylene tubing held in place by a single ligation. The perfused saline passes from the afferent vessel across the lamellae to the efferent vessel, mimicking normal hemolymph circulation, and is collected for analysis. In the intact crab, hemolymph from the ventral sinus is passed through the gills and to the heart by the branchiocardial vessels which open into the pericardial cavity. Tullis described the influence of the factors that decreased water influx and increased sodium

efflux from the isolated perfused gill. Using this technique and automating the system for continuous perfusion and collection of the perfusate with the use of a peristaltic pump and fraction collector, the rate of influx of tritiated water into the gill was estimated. In the green carb *Carcinus maenas* water extracts of ThG perfused through the isolated gill decreased the influx of tritiated water [23]. Similar results were also obtained in *T. crenata*, although a prior extraction of the ThG with acetone was necessary [5] to express the effects of the water soluble fraction in decreasing water influx (Fig. 2). The necessity of a prior extraction with acetone indicates that there are two factors in ThG of crabs acclimated to 100% SW. The activity of the water soluble factor which decreases water influx can also be demonstrated in the hemolymph. Hemolymph extract caused a decrease in water influx in the perfused gill of *T. crenata* (Fig. 3). Acetone extraction prior to water extraction caused a greater effect of the water soluble influx decreasing factor. Apparently, the two factors found in ThG are also present in the hemolymph.

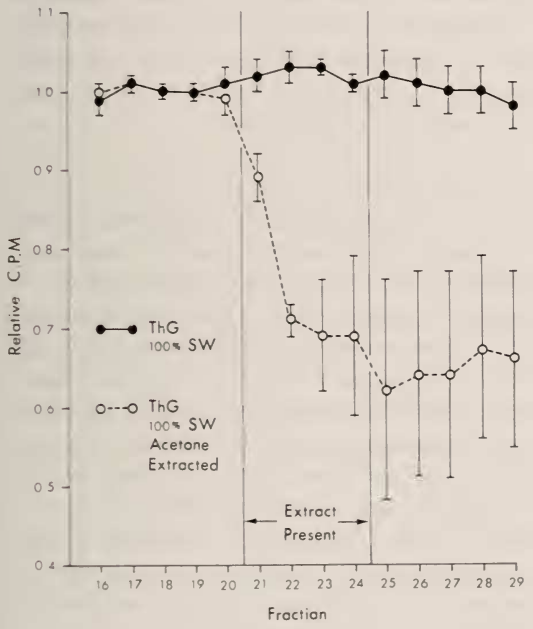

FIG. 2. Effect of thoracic ganglion extract on water influx in the isolated perfused gill of *Thalamita crenata*. Thoracic ganglion is homogenized in deionized water, heated three min in boiling water bath and centrifuged. Supernatant it freeze-dried and reconstituted in crab saline. Osmotic concentration is adjusted to that of normal saline being perfused. Freeze-dried material is extracted with acetone and treated as above for the acetone extracted water soluble fraction. Sampling for influx of trititated water was initiated after equilibration of perfused gill. Extracts were perfused through the gills during periods indicated. Abscissa-sequential 25 drop samples of perfusate. Ordinate-influx values expressed relative to control pre-extract periods as 1.0. Mean ±SE, N=4.

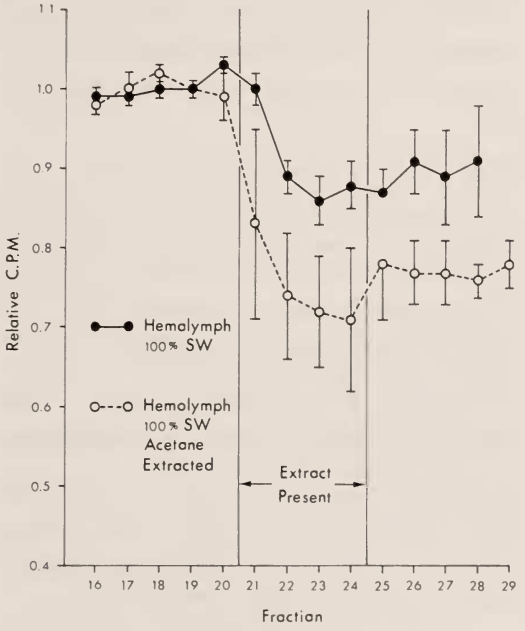

FIG. 3. Effect of hemolymph extract on water influx in the perfused gill of *Thalamita crenata* before and after extraction with acetone. Hemolymph from 100% SW adapted crab is heated 3 min in boiling water bath and centrifuged. Supernatant is freeze-dried and reconstituted with deionized water. Osmotic concentration is adjusted to that of normal saline being perfused. Freeze-dried material is extracted with acetone before reconstitution for acetone extracted water soluble perfusion fluid. Details of perfusion as in Fig. 2.

Similar studies on sodium regulation have been conducted on the perfused gill [24, 25]. When pericardial organ (PO) extracts were perfused through the gills of *Callinectes sapidus*, there was an uptake of sodium by the gill. PO, a neurohemal organ situated in the pericardial cavity, contains five cardioexcitor compounds, three monoamines,

dopamine, octopamine and serotonin, and two peptides [26]. Dopamine causes increases in Na^+ uptake by the gill (Kamemoto, unpublished data). When extracts of hemolymph from crabs adapted to 100% or 25% SW were perfused through the gill, a dramatic increase in Na^+ uptake is seen, suggesting the presence of a PO factor in the hemolymph [25]. The PO factor appears to be dopamine or octopamine. PO extracts and dopamine stimulate Na^+ uptake in intact crabs held in 100% SW [27]. Injections into intact crabs caused significant increases in Na^+ uptake from the medium. No other neuroendocrine tissue extract in known to cause increases in Na^+ uptake in crabs.

Cyclic Nucleotide as Second Messenger

Cyclic nucleotides are believed to be functioning as second messengers for crustacean hormones as the crustacean hyperglycemic hormone [28] and the red pigment dispersing hormone [29]. The nucleotide cAMP appears to be involved in the PO neurohormone activity (Table 1) [24]. Isolated posterior gills of *C. sapidus* were perfused with extracts of ThG and PO, and two components of the PO, dopamine and octopamine. In all cases, there were increases in the cAMP levels of the gills after a 10 min perfusion period. ThG extracts affect water movements while PO extracts and its monoamines increase Na^+ uptake in the gill.

Table 1. The effect of neurohormones on cAMP in the isolated perfused gill of *Callinectes sapidus*. Gills were perfused 10 min with one animal equivalent of Thoracic Ganglion and Pericardial Organ, and 10^{-5} M solutions of Dopamine and Octopamine. Perfusion rates was 0.3 ml/min. N=4. Modified from Kamemoto and Oyama [24]

Experimental Perfusion	Control	Experimental
	pMole cAMP/mg Protein Mean ± SD	
ThG Extract	116.5 ± 25.0	203.4 ± 36.9*
PO Extract	155.7 ± 72.7	312.6 ± 158.5
Dopamine, 10^{-5} M	166.2 ± 38.6	506.4 ± 57.5**
Octopamine, 10^{-5} M	200.5 ± 36.9	408.4 ± 148.1*

* $P < .05$
** $P < .001$

Prefusion of dibutyryl cAMP (dBcAMP) through the isolated gill of *C. sapidus* caused dramatic increases in Na^+ uptake [25]. Injection of dBcAMP into intact crabs also caused increases in Na^+ uptake [27]. Further, incubation of the isolated posterior gills with dBcAMP caused increases in Na^+K^+ATPase activities in the gill [27]. Trausch, et al [30] have suggested that dopamine stimulated adenyl cyclase in the posterior gill of *Eriocheir sinensis,* increasing cAMP and stimulating phosphorylation via a cAMP dependent protein kinase. Clearly, cAMP is activated by neuroendocrine osmoregulators and has a direct effect upon Na^+ uptake in the gill. PO extracts, dopamine, a hemolymph borne factor believed to be dopamine, as well as cAMP induce increased Na^+ uptake by the crab gill. cAMP is a second messenger, mediating the effects of neuroendocrine substances on Na^+ uptake.

Osmotic Stress and Na^+K^+ATPase

Na^+K^+ stimulated, Mg^{2+} dependent and ouabain sensitive ATPase has received considerable attention since the report of Skou [31] of this enzyme in crab nerves. Quinn and Lane [32] reported on the presence of this enzyme in the gills of the terrestrial crab *Cardiosoma guanhumi.* Kato [33] demonstrated this enzyme in the gills of the semiterrestrial crab *Metopograpsus thukuhar.* Na^+K^+ATPase has been recognized as the sodium pump enzyme and has been studied extensively in the posterior gills which are the salt absorbing gills of hyperregulating crabs with specialized cells rich in mitochondria and Na^+K^+-ATPase.

This enzyme appears to be an adaptive enzyme, adjusting to the osmotic stresses placed upon hyperregulating crabs. Increased enzyme activities in the posterior gills of crabs with acute exposure or acclimation to dilute external media have been reported for *Callinectes sapidus* [18, 34], *Thalamita crenata* and *Penopeus herbstii* [19], *Eriocheir japonicus* [35], *Eriocheir sinensis* [36], *Uca pugilator* [37], *Uca pugnax* [38], and *Carcinus maenas* [39]. These crabs are all hyperregulators and it can be generally accepted that exposure to dilute media stimulates increases in Na^+K^+ATPase activity in the posterior gills. This increased enzyme activity

presumably results in greater activity of the sodium pump in the uptake of sodium from a dilute medium.

An anomaly is seen in some semiterrestrial hyperregulators, however. No increase in Na^+K^+-ATPase activity is seen when *Metopograpsus thukuhar* [19], *Uca minax* [37], *Holometopus haematocheir* or *Sesarmops intermedia* [40], all hyperregulating crabs, were exposed to dilute media. There is some evidence that Na^+K^+ATPase activity of gills is increased in hyporegulation when crabs are placed in hyperosmotic media. The Na^+K^+ATPase of anterior gills are increased when *E. japonicus* is placed in 120% SW [35]. Enzyme activity of a gill is dramatically increased when *M. thukuhar* is exposed to 140% SW (Kamemoto and Ueda, unpublished data). Another anomaly is seen in the freshwater carb *Geothelphusa dehaani* [40]. Na^+K^+ATPase is high in the anterior gills with only slight activities in the posterior gills. There is no change in enzyme activity in the gills of *Calappa hepatica,* an osmoconformer, when exposed to various salinities [19].

Neuroendocrine factors are involved in increasing Na^+K^+ATPase activities in the gill, thereby increasing sodium uptake and maintaining salt and water balance. Savage and Robinson [41] observed that the injection of hemolymph collected from *C. sapidus* acclimated to 30% SW into normal 100% SW acclimated crabs caused in increase in gill Na^+K^+ATPase activity within 20 min of injection. This suggested that there is a substance in the hemolymph of crabs acclimated to dilute media which increased enzyme activity and Na^+ uptake from the dilute medium. This effect could not be demonstrated with hemolymph collected from 100% SW acclimated animals. Based on information that PO extracts, dopamine and cAMP increased Na^+ uptake, Sommer and Mantel [27] tested these substances on gill Na^+K^+ATPase activity. Dopamine and PO extracts, injected into *C. sapidus* caused increased Na^+K^+ATPase activities and Na^+ uptake in intact animals. When gills were isolated and incubated with dBcAMP, there was a significant increase in enzyme activity. Dopamine leads to phosphorylation of gill proteins and Na^+K^+ATPase [30] in *E. sinensis*. This adds support to the idea that dopamine might be the hormone secreted by the PO that stimulates Na^+K^+ATPase and the sodium pump in increasing Na^+ uptake by the gills. cAMP appears to be a second messenger in this process.

PO Cardioexcitor Hormones and Osmoregulation

It is clear that the PO and its bioamine are involved in sodium uptake in the posterior gills with the activation of cAMP and Na^+K^+ATPase. Additionally, there is evidence that the PO cardioexcitor hormones may also participate in the overall osmoregulatory process among crabs.

In 1976, Hume and Berlind [42] demonstrated that when the hyperregulating crab *Carcinus maenas* was placed in a dilute medium, heart rate increased. This increased heart rate, assuming that there is no decrease in stroke volume, could increase the circulation of hemolymph through the gills and thereby affect osmoregulation. The authors suggested that the increase in heart rate was caused by the release of PO cardioexcitor hormones in response to exposure of animals to dilute seawater. Osmoreceptors and ionoreceptors, which can perceive changes in external seawater concentrations, are found on the antennules [43, 44] or the anterior portion of the crab [42]. Although the actual release of the cardioexcitor hormones in response to exposure to a dilute medium has not been determined, Zatta [45] was able to demonstrate a rapid increase in dopamine, another component of the PO, in the hemolymph of *C. maenas* after its tranfter to 50% SW.

Cardioexcitation with transfer of crabs into dilute media can be demonstrated in other hyperregulating crabs. With the use of a silver electrode placed on the carapace in the cardiac region and led to a impedence coverter, the heart rate is recorded on a physiograph. One can determine the heart rate of a freely moving crab in a shielded aquarium over an extended period of time [46]. When the heart rate of *C. sapidus* placed in 100% SW is estimated, there is a high rate at the beginning of the experiment which decreases to a steady rate with time (Fig. 4). When the animal is disturbed, as with the changing of the medium, the rate rises and then drops to a new steady rate. When the medium is changed to a dilute SW concentration (15% SW), the new steady heart

rate is higher than that of the control (100% SW) (Fig. 5). Using this procedure, a steady state heart rate for a single individual after several transfers in SW concentrations can be established. This increase in heart rate in response to a dilute medium is controlled by cardioexcitor hormones of the PO and could possibly effect greater osmorgulatory activities in the gills.

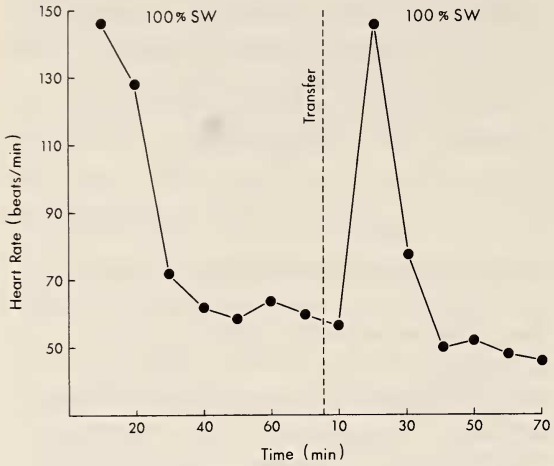

FIG. 4. Heart rate of *Callinectes sapidus* in 100% SW.

Conclusions

Neuroendocrine substances regulate water and sodium movements into the posterior gills of hyperregulating crabs. A ThG water soluble factor, presumably a peptide with a molecular weight of 800–1000 daltons, decreases water influx while an acetone soluble factor increases water influx. A PO hormone, dopamine, increases sodium uptake. Cyclic AMP is a probable second messenger.

Gill Na^+K^+ATPase is an adaptive enzyme in osmoregulating crabs. This enzyme is increased when crabs are exposed to dilute media, stimulating an increase in sodium uptake by the gills. Dopamine stimulates increase in gill cAMP and Na^+K^+ATPase and affects the cAMP dependent protein kinase in the gill.

Increased heart rate may also contribute to osmoregulation by increasing hemolymph flow through the gills. PO cardioexcitor hormones increase heart rate in response to the crab's exposure to a dilute medium.

The following neuroendocrine adaptation process in hyperregulation among crabs is suggested. As a hyperregulating euryhaline crab ventures into estuarine and freshwaters, receptors, found on the antennules, perceive changes in the osmotic and ionic concentrations of the water. The ThG releases a water soluble peptide which decreases the osmotic influx of water to aid in the maintenance of osmotic concentration of the hemolymph. Additionally, the PO releases dopamine and cardioexcitor peptides. Dopamine increases sodium uptake by the gills by the activation of the cAMP

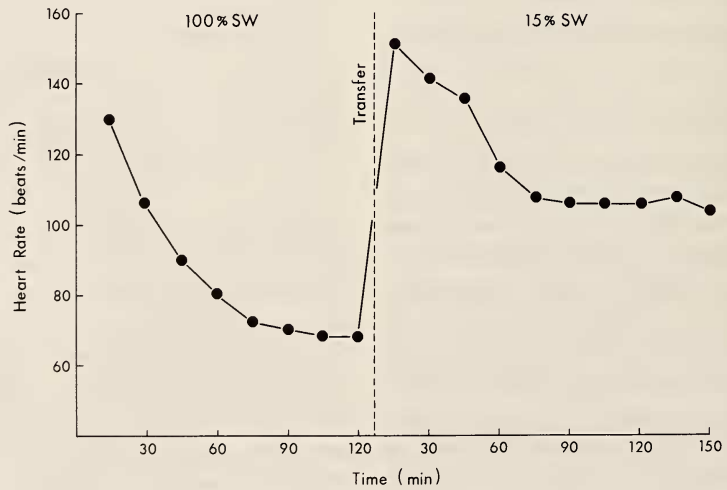

FIG. 5. Heart rate of *Callinectes sapidus* when transferred from 100% to 15% SW.

second messenger system. Na^+K^+ATPase activity is also increased. The cardioexcitor peptides increase heart activity and hemolymph circulation through the gills which facilitate osmoregulation. All of these activities appear to be involved in neuroendocrine integration of osmoregulation in crabs.

REFERENCES

1 Kamemoto, F. I., Kato, K. N. and Tucker, L. E. (1966) Am. Zool. **6**: 213–219.
2 McWhinnie, M. A. (1962) Comp. Biochem. Physiol., **7**: 1–14.
3 Kamemoto, F. I. and Tullis, R. E. (1972) Gen. Comp. Endocrinol. Suppl., **3**: 299–307.
4 Kamemoto, F. I. (1976) Am. Zool., **16**: 141–150.
5 Kamemoto, F. I. (1982) In "Neurosecretion: Molecules, Cells, Systems" pp. 329–335. Ed. by D. S. Farner and K. Lederis Plenum, New York.
6 Mantel, L. H. and Farmer, L. L. (1983) In "Biology of Crustacea" Vol. 5 pp. 54–161. Ed. by L. H. Mantel, Academic Press, New York.
7 Mantel, L. H. (1985) Am. Zool., **25**: 253–263.
8 Kamemoto, F. I. and Kato, K. N. (1969) Pac. Sci., **23**: 232–237.
9 Tullis, R. E. and Kamemoto, F. I. (1974) Gen. Comp. Endocrinol., **23**: 19–28.
10 Koch, H. J., Evans, J. and Schicks, E. (1954) Medel. Kon. VI Acad. Kl. Wet., **16**: 1–16.
11 Croghan, D. C., Curra, R. A. and Lockwood, A. P. M. (1965) J. Exp. Biol., **42**: 463–474.
12 Gilles, R., Pequeux, A. and Bianchini, A. (1988) Comp. Biochem. Physiol., **90A**: 201–207.
13 Towle, D. W. (1990) In "Comparative Physiology" Ed. by R. Kinne, Karger Publishing, Basel.
14 Burnett, L. E. and Towle, D. W. (1990) J. Exp. Biol., **149**: 293–305.
15 Lucu, C. (1990) Comp. Biochem. Physiol., **97A**: 297–306.
16 Copeland, D. E. and Fitzjarrell, A. T. (1968) Z. Zellforsch., **92**: 1–22.
17 Goodman, S. H. and Cavey, M. J. (1990) Cell Tiss. Res., **260**: 495–505.
18 Mantel, L. H. and Olson, J. R. (1976) Am. Zool., **16**: 223.
19 Spencer, A. M., Fielding, A. H. and Kamemoto, F. I. (1979) Physiol. Zool., **52**: 1–10.
20 Neufeld, G. J., Holliday, C. W. and Pritchard, J. B. (1980) J. Exp. Zool., **211**: 215–224.
21 Towle, D. W. and Kays, W. T. (1986) J. Exp. Zool., **239**: 311–318.
22 Tullis, R. E. (1975) Am. Zool., **15**: 786.
23 Berlind, A. and Kamemoto, F. I. (1977) Comp. Biochem. Physiol., **58A**: 383–385.
24 Kamemoto, F. I. and Oyama, S. N. (1985) In "Current Trends in Comparative Endocrinology" pp. 883–886. Ed. by B. Lofts and W. N. Holmes, Hong Kong University Press, Hong Kong.
25 Lohrmann, D. M. and Kamemoto, F. I. (1987) Gen. Comp. Endocrinol., **65**: 300–305.
26 Cooke, I. M. and Sullivan, R. E. (1982) In "The Biology of Crustacea" Vol. 3 pp. 206–291. Ed. by H. L. Atwood and D. C. Sandeman, Academic Press, New York.
27 Sommer, M. J. and Mantel, L. H. (1988) J. Exp. Zool., **248**: 272–277.
28 Sedlmeier, D. and Keller, R. (1981) Gen. Comp. Endocrinol., **45**: 82–90.
29 Fingerman, M. (1969) Am. Zool., **9**: 443–452.
30 Trausch, G., Forget, M.-C. and Devos, P. (1989) Comp. Biochem. Physiol., **94B**: 487–492.
31 Skou, J. C. (1957) Biochem. Biophys. Acta, **23**: 394–402.
32 Quinn, D. J. and Lane, C. E. (1966) Comp. Biochem. Physiol., **19**: 533–543.
33 Kato, K. N. (1968) Ph. D. Thesis, University of Hawaii, Honolulu, Hawaii.
34 Towle, D. W., Palmer, G. E. and Harris, III J. L. (1976) J. Exp. Zool., **196**: 315–322.
35 Watanabe, K. and Yamada, J. (1980) Bull. Fac. Fish. Hokkaido Univ., **31**: 283–289.
36 Pequeux, A., Marchal, A., Wanson, S. and Gilles, R. (1984) Mar. Biol. Letters **5**: 35–45.
37 D'orazio, S. E. and Holliday, C. W. (1985) Physiol. Zool., **58**: 364–373.
38 Holliday, C. W. (1985) J. Exp. Zool., **233**: 199–209.
39 Wiebers, D., Winkler, A., Lucu, C., Thedens, G. and Weichart, D. (1985) Mar. Biol., **87**: 185–192.
40 Kamemoto, F. I. and Deguchi, Y. (1986) Am. Zool., **26**: 91A.
41 Savage, J. P. and Robinson, G. D. (1983) Comp. Biochem. Physiol., **75A**: 65–69.
42 Hume, R. I. and Berlind, A. (1976) Biol. Bull., **150**: 241–254.
43 Well, P. B. van and Christofferson, J. P. (1966) Physiol. Zool., **39**: 317–325.
44 Weel, P. B. van and Correa, L. H. (1967) Zool. Jb. Physiol., **73**: 174–185.
45 Zatta, P. (1987) Mar. Biol., **96**: 479–481.
46 Galinato, E. P. and Kamemoto, F. I. (1983) 11th Ann. MBRS Symposium: 144.